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13. ABSTRACT ***(Maximum 200 Words)***

To test our hypothesis that progesterone induces focal adhesion in breast cancer cells through its regulation of IRS-1/2 expression and activation, I first determined whether progesterone regulated focal adhesion in different breast cancer cell lines with distinct progesterone receptor (PR) and insulin receptor substrate (IRS)-1/2 levels. Preliminary analysis using Texas-red-phalloidin and fluorescence microscopy showed that stress formation was induced in PR-B transfected C4-12 cells (ER-/PR- MCF-7 sublines). To assess whether progestins induce cell adhesion through regulation of IRS-1/2 expression and activation, I performed RT-PCR and western blot analysis. It was found that IRS-2 was sharply up-regulated by progestins in MCF-7, T47D, and especially in PR-B C4-12 cells while IRS-1 was only slightly induced. This indicates that IRS-1 and IRS-2 are distinctively regulated by progestins. Co-treatment of R5020 with the transcription inhibitor 5,6-dichlorobenzimidazole riboside (DRB) blocked the induction of IRS-2, suggesting that the progestin effect was mediated by a transcriptional mechanism. These data provide a clue that IRS-2 may be involved in progestin-induced cell adhesion as IRSs have been shown to interact with many cell adhesion proteins.

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Introduction

Progesterone is involved in breast cancer development. It has been proposed that progesterone primes breast cancer cells for the actions of growth factors and cytokines signals (Richer et al. 1998). Insulin receptor substrate (IRS) proteins are important signaling integrators which can associate with different proteins and subsequently activate various downstream signaling pathways following stimulation of insulin, insulin-like growth factors (IGFs), other growth factors and cytokines (Bruning et al. 1997). IRS molecules are also implicated in breast cancer cell growth (Nolan et al. 1997). Interestingly, progesterone was shown to up-regulate IRS-2 levels in the progesterone receptor (PR)-B-transfected HeLa cells (Vassen et al. 1999). How progesterone and IRS signaling pathways cross-talk with each other to regulate breast cancer progression remains a critical question.

Recently, it was reported that progesterone induces focal adhesion in MDA-MB-231 breast cancer cells transfected with PR cDNA (Lin et al. 2000). In T47D cells, progestins induce the expression of desmoplakins (Kester et al. 1997), which are essential intracellular attachment proteins that connect intermediate filaments with desmosomes, which in turn interact with transmembrane linker proteins to bind the membranes of adjacent cells. These findings suggest that progesterone promotes cell adhesion. Opposite to the progesterone effect, IGF-IR was found to reduce focal adhesion in breast cancer cells characterized by actin filament disassembly and tyrosine dephosphorylation of focal adhesion proteins (Guvakova and Surmacz 1999). IGF-I was previously demonstrated to stimulate chemotaxis of breast cancer cells (Doerr and Jones 1996). Interestingly, it has been reported that progesterone down-regulates the IGF-IR mRNA levels in normal breast tissues and breast cancer cells (Goldfine et al. 1992; Clarke et al. 1997). Hence, it is reasonable to speculate that progesterone induces focal adhesion by altering IGF signaling. As a major substrate of IGF-IR, IRS-1 was found to interact with proteins involved in focal adhesion and stress fiber formation (Lebrun et al. 1998). Based on these rationales, I propose a hypothesis that progesterone induces focal adhesion in breast cancer cells through its regulation of IRS-1/2 expression and activation. In this study, I showed that the progestin R5020 increased IRS-1/ 2 expression in breast cancer cells.

Body

1. To determine whether progesterone regulates focal adhesion in different breast cancer cell lines with distinct PR levels

As a first to investigate the potential role of IRS-1/2 in the progesterone effect on cell adhesion, I examined whether progesterone had the same effect in normal breast cancer cell lines MCF-7 and T47D as in the PR-B-transfected MDA-MB-231 cells. As is well known, MCF-7 cells high IRS-1 levels, low IRS-2 levels, and moderate PR levels while T47D cells have moderate IRS-1 levels, low IRS-2 levels, and high PR levels. Since progesterone has a short half-life in cells, I utilized the synthetic progestin R5020 as a substitute. Using phase contrast light microscopy, it was found that the progestin R50202 treatment of MCF-7 and T47D cells did not cause significantly visible cell spreading and schange of cell morphology. It was also found that stress fiber formation was not induced by progestin treatment using Texas red-phalloidin and fluorescence microscopy. Since these two cell lines have considerable expression of ER which was shown to inhibit cell adhesion and stress fiber formation, and may mask the effect of

progestin, it is desirable that ER-/PR+ cell lines may be appropriate for the optimal induction of the progestin effect on cell adhesion. Thus, PR-A and PR-B were stably transfected into a specially selected MCF-7 cell line C4-12 which is ER-/PR- but still has considerable IRS-1/2 levels (Oesterreich et al. 2001). Preliminary analysis showed that stress fiber formation was enhanced in the PR-B-transfected C4-12 cells by progestin treatment, which is consistent with the result from PR-B transfected MDA-MB-231 cells (Lin et al. 2000). I am currently confirming this data.

2. To analyze whether progesterone regulation of focal adhesion correlates with changes of IRS-1/2 expression and activation

To assess whether progestins affect IRS-1/2 expression in breast cancer cells, I first performed western blot analysis using MCF-7 and T47D cells. It was found that the progestin R5020 slightly or moderately increase IRS-1 levels in these cells, while markedly elevate IRS-2 levels. Then I tested the progestin effect in PR-A/B transfected C4-12 cells. As expected, IRS-1/2 levels were only altered in PR-B C4-12 cells, which reflected the accepted notion that PR-B is the major mediator of progestins. It was found that IRS-1 expression was slightly enhanced in PR-B C4-12 cells. However, IRS-2 was sharply up-regulated in these cells, which was more dramatic than MCF-7 and T47D cells. This may be due to the fact that PR-B C4-12 cells have higher PR-B levels than MCF-7 and T47D cells, and PR-A may impair the function of PR-B. This is an interesting result, as a recent report showed that IRS-2 regulates breast cancer cell motility in metastatic variants of human breast cancer cell lines (Jackson et al. 2001). Time course experiment using 10 nM R5020 showed that 6 h treatment of R5020 caused a detectable increase of IRS-2 levels which was markedly elevated at 12 h and at 24 h the induction of IRS-2 plateau out. Dose response assay displayed that the maximal induction of IRS-2 occurs at 10^{-10} M R5020.

Next, I evaluated whether this R5020 effect on IRS-2 was mediated through transcriptional mechanism. RT-PCR was performed using 2 μg total RNA and a pair of primers at the C-terminus of the IRS-2 coding region. It was found that in PR-B C4-12 cells IRS-2 mRNA were dramatically up-regulated by R5020 consistent with the immunoblotting result. Pre-treatment with the specific transcription inhibitor 5,6-dichlorobenzimidazole riboside (DRB) before R5020 stimulation blocked the increase of IRS-2 expression, suggesting that the progestin effect on IRS-2 is mediated by a transcriptional mechanism. I am currently testing changes of tyrosine phosphorylation levels of IRS-2 by co-treatment of IGF-I and R5020.

Interestingly, during our study of progestin regulation of IRSs, we also found that IGF-I itself vice versa regulated PR. IGF-I treatment of MCF-7 cells sharply lowered PR levels and corresponding progestin-induced PR activity. This effect is mediated by PI-3 kinase/Akt pathway through a transcriptional mechanism. This finding provides new insight into the cross-talk between progestin and growth factors.

3. To test the involvement of IRS-1/2 in focal adhesion induction by progesterone in breast cancer cells

This aim has not been studied yet.

Key research accomplishments

- Generation of PR-B C4-12 cells. C4-12 cells were derived from MCF-7 cells and are ER-/PR-. Thus the influence of ER can be ruled out in our study.
- Demonstration of IRS-2 induction by progestins in MCF-7, T47D, and PR-B C4-12 cells. This effect is mediated through transcriptional mechanisms.

Reportable outcomes

- Poster presentation "Progestins induce insulin receptor substrate-1 and -2 expression in breast cancer cells" at the 24th annual San Antonio Breast Cancer Symposium, December 10-13, 2001.
- Poster presentation "Progestins induce insulin receptor substrate-1 and -2 expression in breast cancer cells" at the 83rd annual meeting of the Endocrine Society, June 20-23, 2001

Conclusions

Progestins significantly up-regulate IRS-2 expression in breast cancer cells. This suggests that progestin induced cell adhesion may involve IRS-2.

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